

SALMEN[®] – A COMPLEXATION REAGENT FOR Cr (III)

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Abstract

The biological activity of chromium (essential oligoelement) depends on its valence and on the chemical structure of the formed complex combinations. The present spectrophotometric method has been established for the quantitative assay of Cr (III), and it is based on its complexation with N,N'-bis (salicyliden)-methylenediamine (noted Salmen[®]), when a stable complex is formed, which has a maximum of absorption at 326 nm. The developed method has been validated and was characterized by a good linearity in the range of 0.5–5.0 µg/mL (correlation coefficient $r = 0.9999$). The established detection limit (LOD) was 0.066 µg/mL and the quantification limit (LOQ) was 0.22 µg/mL. There were determined the precision of the method (RSD = 1.63%) and the accuracy (the mean recovery was established at 100.17%, the recovery values being in the range 98.0–102.3 %). The proposed and validated method was applied for the determination of Cr(III) from a nutritional supplement that contained the salt chromium picolinate, and the results were good.

Rezumat

Activitatea biologică a cromului (oligoelement esențial) depinde de valența sa și de structura chimică a combinațiilor complexe formate. Pentru determinarea cantitativă a cromului s-a elaborat o metodă spectrofotometrică ce constă în complexarea lui cu N,N-bis (saliciliden)-metilendiamina (notat Salmen[®]) cu formarea unui complex stabil ce prezintă un maxim de absorbție la 326 nm. Metoda propusă a fost validată și prezintă o bună liniaritate în domeniul 0,5–5,0 µg/mL (coeficientul de corelație $r = 0,9999$). Limita de detecție găsită este 0,066 µg/mL și limita de cuantificare 0,22 µg/mL. S-au determinat precizia metodei (RDS = 1,63%) și exactitatea (regăsirea medie fiind de 100,17% pe domeniul 98,0–102,3%). Metoda propusă și validată, a fost aplicată la determinarea Cr(III) din suplimentul alimentar ce conține sarea picolinat de crom cu bune rezultate.

Keywords: Cr (III), spectrophotometry, validation, applications.

Introduction

Chromium is a biometal known as a redox catalyst, an oxygen transporter and as Cr (III) an essential nutritive agent. In the case of patients with insulin-dependent diabetes, it has been noticed an abnormal metabolism of Cr(III), while Cr (VI) ions that result from various industrial

processes such as: coloring matter industry, metal, steel and alloys processing, are toxic, mutagen and carcinogen [4, 8].

Cr(III) can be determined quantitatively directly through an UV spectrophotometric method. We have determined the best conditions in which the complexation reaction takes place using as reagent the Schiff bis base ONNO type (Salmen[®]) [1]: optimum pH, formation time, complex stability in time, combination ratio M/L, stability conditional constant (β_n) and interferences [7]. This paper refers to the validation of the UV spectrophotometric method for the determination of the Cr(III) ions [2, 3, 5] and its application on the determination of Cr(III) from the food supplement Crom Forte[®] 200 µg tablets produced by Walmark (Czech Republic).

The validation of the spectrophotometric method was performed in accordance with International Conference on Harmonization guidelines (ICH) [10].

Materials and methods

Principle of the method: the Cr(III) ions, in the presence of N,N'-bis (salicyliden)-methylenediamine (Salmen[®]), at pH=4.6, form a complex with a maximum at 326 nm, that is proportional to the concentration of the analyzed ions. There were established the optimum wavelength for the detection and the optimum working conditions: optimum pH, formation time, complex stability in time, combination ratio M/L, stability conditional constant (β_n), interferences [7]. In order to evaluate the performance parameters for the method (linearity, precision, accuracy and standard calibration), solutions in the concentration range 0.5-5.0 µg/mL Cr(III) were used.

Reagents and apparatus

- stock solution of Cr(III) 0.1 mg/mL: 0.04753g Cr(CH₃COO)₃·H₂O (Carlo Erba) were dissolved in 100 mL distilled water;
- work solutions with concentrations ranging between 0.5-5.0 µg/mL Cr(III), obtained by diluting the stock solution with distilled water;
- N,N'-bis (salicyliden)-methylenediamine (Salmen[®]) - 0.01% solution (w/v) prepared using pure methanol (Merck) – reagent solution;
- acetic acid (Merck) – sodium acetate (Merck) buffer solution 0.2M (pH=4.6);
- Crom Forte[®] 200 µg tablets produced by Walmark (Czech Republic);
- spectrophotometer UV-VIS – HEWLETT–PACKARD 8453.

Establishing the optimum wavelength for the detection.

Procedure: 1 mL solution to be analyzed containing 0.5–5.0 µg/mL Cr(III) was treated with 1 mL buffer solution (pH=4.6), 2 mL absolute methanol and 1 mL reagent solution 0.01% (w/v) in methanol. After 10 minutes, the extinction was measured at 326 nm and it was compared to a blank sample, prepared using 3 mL of methanol, 1 mL of buffer solution (pH=4.6) and 1 mL of reagent solution. Also, the optimum working conditions were established.

Method validation:

Linearity: In order to determine the linearity of the method we prepared Cr(III) solutions by diluting the stock solution with distilled water in the 0.5–5.0 µg/mL concentration range. The obtained data was analyzed by linear regression and the calibration curve was obtained [3, 6, 9].

Detection and quantification limits were calculated using the following formulas [6]:

$$\text{LOD} = 3 \cdot \text{Standard error} / \text{slope}; \quad \text{LOQ} = 10 \cdot \text{Standard error} / \text{slope}.$$

Precision [3,6,9]: three solutions of 1.5, 2.0, 2.5 µg/mL Cr(III) ions were used. Three assays were performed for each concentration. Two sets of assays were performed in different days in order to evaluate the intermediary precision.

Accuracy [3,6,9]: in order to establish the accuracy of the method for Cr(III) determination there were used the following three solutions 1.5, 2.0, 2.5 µg/mL. For each solution, three determinations were performed.

Results and discussion

The absorption spectrum of the Salmen[®]-Cr(III) complex (Figure 1) shows a maximum of absorbance at 326 nm, when compared to Salmen[®] itself, that has a maximum of absorption located at 299 nm.

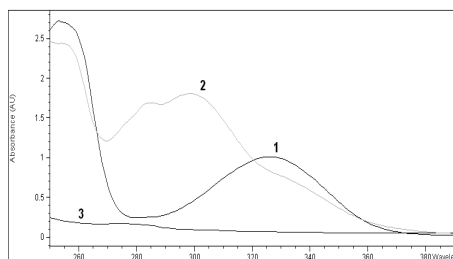


Figure 1

Absorption UV spectra for
1. Salmen[®]-Cr(III) complex;
2. Salmen[®]; 3. Cr(III).

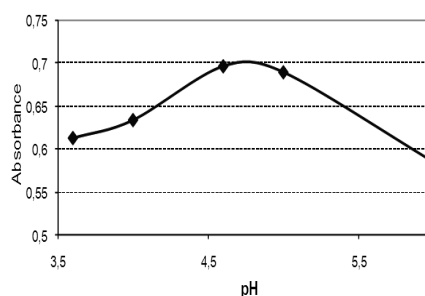


Figure 2

The influence of pH on the
complexation reaction

The pH value influences the complexation reaction and the optimum pH was established at 4.6 (Figure 2).

The complex formed within 10 minutes and it was stable for another 15 minutes, as it is shown in Figure 3. The combination rate was established by the isomolar series method and is illustrated in Figure 4.

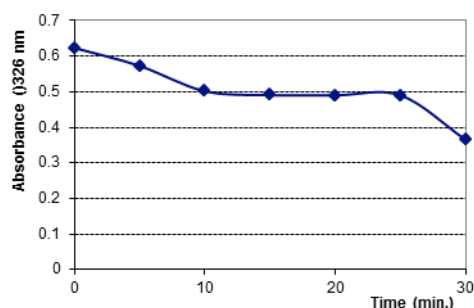


Figure 3
Complex stability in time

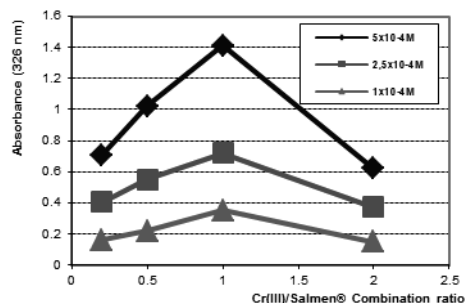


Figure 4
M/L Molar ratio

Stability conditional constant (β_n) was established according to the formula:

$$\beta_n = (\log C_M \cdot C_L) / (\log A - n \cdot \log V) \quad [7],$$

where: C_M = molar metal concentration (Cr(III) ions);

C_L = molar ligand concentration (Salmen[®]);

A = metal-ligand complex absorbance measured at 326 nm (Salmen[®] - Cr(III) complex);

n = M/R molar ratio (Cr(III)/Salmen[®] combination ratio);

V = the volume of the solution (mL);

$\epsilon = 1.4 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$ molar extinction coefficient.

According to the data collected, the obtained value was $\beta_n = 7.17 \cdot 10^6$.

Linearity

For the study of the linearity of the method, we prepared four series (I-IV) of Cr(III) solutions in the 0.5-5.0 $\mu\text{g/mL}$ concentration range. The obtained data were statistically evaluated (table I) and the calibration curve was obtained (figure 5).

Table I
Linearity of the method

No	Concentration (µg/mL)	Absorbance (326 nm)				
		I	II	III	IV	Mean
1	0.5	0.16541	0.18563	0.17428	0.17676	0.17552
2	1.0	0.24376	0.23354	0.23911	0.23819	0.23865
3	1.5	0.29084	0.30710	0.29992	0.29796	0.29897
4	2.0	0.35963	0.36169	0.36036	0.36096	0.36066
5	2.5	0.41578	0.43032	0.42375	0.42235	0.42305
6	3.0	0.48851	0.49473	0.49116	0.49208	0.49162
7	3.5	0.54602	0.54824	0.53924	0.55502	0.54713
8	4.0	0.61021	0.62043	0.61476	0.61588	0.61532
9	4.5	0.66819	0.67625	0.67168	0.67275	0.67222
10	5.0	0.72462	0.74088	0.73221	0.73329	0.73275
Absorbance = $0.1244 \times \text{Concentration} + 0.1136$, Correlation and regression coefficients: $r = 0.9999$, $r^2 = 0.9998$, Standard error = 0.00275, Intercept = 0.113596 ± 0.001879 , Slope = 0.124361 ± 0.0000606 .						

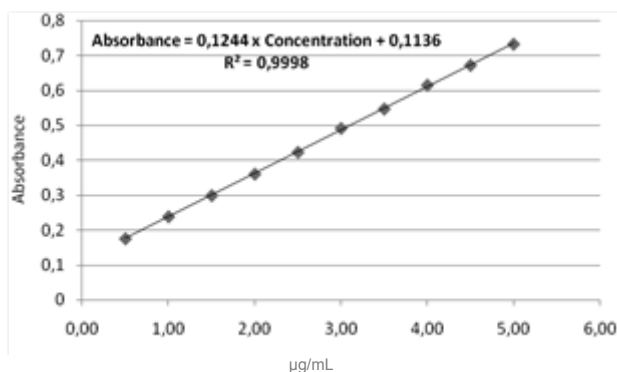


Figure 5

The calibration curve developed for the linearity of the method

The detection limit (LOD) and the quantification limit (LOQ) were calculated: $\text{LOD} = 3 \times 0.00275/0.1244 = 0.066 \text{ µg/mL}$ and $\text{LOQ} = 10 \times 0.00275/0.1244 = 0.22 \text{ µg/mL}$.

Precision

We calculated the sample concentration using the calibration curve equation (table I). We observed that for each set of data the relative standard deviation was lower than 2% ($\text{RSD} = 1.63\%$). All these values confirmed that the proposed method is precise.

Accuracy

The concentration of the sample was calculated from the experimental value of the absorbance, using the regression curve equation (table II). We observed that the recovery was 100.17% for the studied concentration range, the mean (minimum was 98.00% and maximum was 102.03%) and the relative standard deviation was under 2% (RSD=1.23%). These values proved that the Cr(III) determination method is accurate.

Table II
The method precision and accuracy

Cr(III) (µg/mL)	Method precision		Intermediate precision		Accuracy	
	Absorbance	Recovery %	Absorbance	Recovery %	Absorbance	Recovery %
1.5	0.30393	102.05	030393	102	0.29897	99.34
	0.30268	101.52	0.30766	104	0.29902	99.68
	0.29895	99.34	0.29896	99.33	0.30297	101.50
2.0	0.36302	101.55	0.36862	102.5	0.36825	102.30
	0.37359	103.22	0.37359	104.5	0.36285	100.18
	0.36196	99.82	0.36116	99.5	0.36196	99.82
2.5	0.43455	103.25	0.43082	102	0.41838	98.00
	0.43330	103	0.42709	100.8	0.42592	100.42
	0.43082	102	0.42336	99.6	0.42558	100.31
Statistical Data	Mean Recovery = 101.75 % RSD = 1.38%		Mean Recovery = 101.58 % RSD = 1.89%		Mean Recovery = 100.17 % RSD = 1.23%	

Application

The validated method was applied to the UV spectrophotometric assay of Cr(III) from Crom Forte[®] 200 µg tablets produced by Walmark (Czech Republic). The content (µg) of Cr(III) per tablet, was calculated using the formula:

$$\mu\text{g Cr(III)} / \text{tablet} = [(A - 0.1136) / 0.1244] \cdot V \cdot \text{MW} / a,$$

where: A = sample absorbance measured at 326 nm;

V = the capacity of the volumetric flask (mL);

MW = mean weight of the tablets (g);

a = processed sample weight (g).

The obtained results are presented in table III.

Table III
Spectrophotometric assay of Cr(III)

Analyzed Product	Certified quantity of Cr(III) (µg/tablet)	Recovered Cr(III) (µg/tablet)	Recovery (%)	RSD (%)
Crom Forte [®] (chromium picolinate)	200	198.55±1.175	99.27	1.47

Conclusions

A new spectrophotometric method for the assay of Cr(III) was developed, based on Cr(III) complexation reaction with Salmen[®]. The complex presents a maximum of absorption at 326 nm. The analyzed method has been validated, establishing the optimum wavelength of detection (326 nm), the linearity (in the range of the 0.5–5.0 µg/mL, the correlation coefficient being $r=0.9999$), the detection limit (LOD=0.066 µg/mL), the quantification limit (LOQ=0.22 µg/mL), the precision of the method (RSD=1.63%) and the accuracy (mean recovery=100.17%).

In conclusion, the proposed method is linear, precise, accurate, simple and fast, and it was used, with good results, for the quantitative assay of Cr(III) from Crom forte[®] 200 µg tablets produced by Walmark (Czech Republic).

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